FORUM

Sequencing a New Target Genome: The *Boophilus microplus* (Acari: Ixodidae) Genome Project

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ABSTRACT The southern cattle tick, *Boophilus microplus* (Canestrini), causes annual economic losses in the hundreds of millions of dollars to cattle producers throughout the world, and ranks as the most economically important tick from a global perspective. Control failures attributable to the development of pesticide resistance have become commonplace, and novel control technologies are needed. The availability of the genome sequence will facilitate the development of these new technologies, and we are proposing sequencing to a 4–6X draft coverage. Many existing biological resources are available to facilitate a genome sequencing project, including several inbred laboratory tick strains, a database of ≈45,000 expressed sequence tags compiled into a *B. microplus* Gene Index, a bacterial artificial chromosome (BAC) library, an established *B. microplus* cell line, and genomic DNA suitable for library synthesis. Collaborative projects are underway to map BACs and cDNAs to specific chromosomes and to sequence selected BAC clones. When completed, the genome sequences from the cow, *B. microplus*, and the *B. microplus*-borne pathogens *Babesia bovis* and *Anaplasma marginale* will enhance studies of host–vector–pathogen systems. Genes involved in the regeneration of amputated tick limbs and transitions through developmental stages are largely unknown. Studies of these and other interesting biological questions will be advanced by tick genome sequence data. Comparative genomics offers the prospect of new insight into many, perhaps all, aspects of the biology of ticks and the pathogens they transmit to farm animals and people. The *B. microplus* genome sequence will fill a major gap in comparative genomics: a sequence from the Metastriata lineage of ticks. The purpose of the article is to synergize interest in and provide rationales for sequencing the genome of *B. microplus* and for publicizing currently available genomic resources for this tick.

KEY WORDS *Boophilus microplus*, genome sequence, gene identification, *Babesia bovis*, molecular genetics

Boophilus ticks are present in many parts of the world with *B. microplus*, the tropical or southern cattle tick, being the most widely distributed species and the most important economically. Originally from Asia, this one-host tick species has colonized most of the world's tropical and subtropical countries (McCosker 1979, Murrell et al. 2001). These ticks transmit protozoan (*Babesia bovis* and *Babesia bigemina*) and prokaryotic (*Anaplasma marginale*) organisms that cause babesiosis and anaplasmosis. The tick–disease complex of *Boophilus* spp.–*Babesia* spp.–*Anaplasma marginale* is probably the most important complex affecting worldwide agriculture (de Castro 1997), leading to severe economic losses in milk and beef production and restriction in traffic of livestock.

B. microplus has rapidly developed acaricide resistance in Africa, Australia, and North and South America. In many countries, the resistance phenotype extends to several chemical classes such as arsenicals, organochlorines, organophosphates, amidines, macrocyclic lactones, and pyrethroids (Angus 1996, Kemp et al. 1998, Benavides et al. 1999, Miller et al. 1999) with severe economic consequences for cattle producers. For example, in Australia, the tick has acquired resistance to pyrethroid, organophosphate, and amitraz acaricides, resulting in reported annual losses of $100 million per year attributable to *B. microplus* (Angus 1996). Brazil has a cattle population of ≈170 million. Producers of that country incur direct and indirect losses of >$2 billion attributable to ticks and tick control expenditures. Determination of the genetic basis of resistance to acaricides has been elusive despite over a decade of research (Baxter and Barker 1998, 1999a, b, 2002; Hernandez et al. 1998, 2002; Baxter et al. 1999; Crampton et al. 1999; Jamroz et al. 2000; Guerrero et al. 2002; Pruett et al. 2002; Temeyer et al. 2004).

The inadvertent importation of tick-infested cattle into the United States around the early part of the nineteenth century resulted in the rapid establish-

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ment of *B. microplus* ticks in 14 southern states and southern California and the concurrent transmission of bovine babesiosis (Graham and Hourrigan 1977). The cattle industry's annual losses attributable to *Boophilus* ticks were estimated in 1906 to be $130,500,000 and led to a federal–state *Boophilus* eradication program initiated in 1906. The program was essentially completed by 1943, but the ticks were not eliminated from Florida until 1960, and outbreaks still occur in Texas, generally confined to a buffer zone ≈500 miles in length and 0.25 to 10 miles in width along the U.S.–Mexico border (Graham and Hourrigan 1977).

Because of the widespread prevalence of pathogen-infected *Boophilus* ticks in Mexico, the eradication status is maintained using a 500-mile "buffer zone" along the U.S.–Mexico border with mandatory acaricide treatment of livestock before importation into the United States (Bram et al. 2002, George et al. 2002). Annual costs for this program are ≈$4 million (Dietrich and Adams 2000). The development of *B. microplus* populations resistant to multiple acaricides, particularly acaricides approved for use in the border buffer zone treatment vats, is a major risk factor for reintroduction and reestablishment of this vector in the continental United States. Genomic sequence data for *B. microplus* should enhance the potential for the identification of biological targets for developing novel chemotherapeutic chemistries to effect tick control strategies complementing current acaricide-based control methods.

The feasibility of controlling *B. microplus* through use of a recombinant vaccine was shown by the commercial release of such a vaccine in 1994 (Willadsen et al. 1995). The limitations of the current vaccine are also well known. Its principal effect is to reduce the fecundity of ticks, which means it is not suitable for the very high level of tick control required to keep the United States *Boophilus*-free. Experimentally, it has been shown that a vaccine can have efficacy equivalent to that of a chemical acaricide (Fragoso et al. 1998). Improvement of the existing vaccine to reach this standard, so that it meets U.S. requirements as well as establishing a global market for a vaccine, demands both a better understanding of tick biology and an efficient identification of tick antigens. These aims would be greatly facilitated by a genome sequence. This article presents the rationale for obtaining a draft quality sequence of *B. microplus* in light of the potential contributions the sequence would afford researchers in a number of fields. Additionally, we publicize the available genetic resources and ongoing projects on the genomics of *B. microplus.*

Specific Biological Rationales for the Utility of New Sequence Data

Improving Human Health. Ticks are thought to be among the most ancient terrestrial arachnids and possibly the earliest to evolve blood-feeding capabilities (reviewed in Mans and Neitz 2004). The evolution of this capability likely occurred independently in response to the evolution of diverse speciation in higher animals, resulting in the acquisition of antihemostatic capabilities that differ from one another across genus level. The dearth of arachnid genome sequence presents an opportunity to explore genes with potentially novel functions. Host response to the tick bite involves hemostasis, inflammation, and both innate and acquired immunity. Because *B. microplus* is a one-host tick that must maintain sustained (3-wk) contact with its host, it has developed a unique means of avoiding the host animal's responses to the bite (Wikel 1999), and extracts of its salivary glands have been shown to have an immunosuppressive effect on the bovine host (Turni et al. 2002, 2004). The tick also must respond to many microorganisms, both symbiotic and parasitic. Sequence data from *B. microplus* is expected to yield information on gene-based responses to these stresses that could lead to discovery of novel antibiotics, immunologicals, and other classes of biopharmaceutical or bioactive substances. Numerous inhibitors of the mammalian hemostatic system have been described in ticks, and several biomolecules derived from tick saliva have already been tested for potential use as human pharmaceuticals (P.A. Nuttall, unpublished data).

Without genome sequences from both lineages of hard ticks, the Prostriata, which consists of the single genus *Ixodes* containing ≈250 species, and Metastriata, which consists of >400 species from several genera, including *B. microplus* (Tree of Life Web Project, http://tolweb.org/tree?group = Ixodida&contgroup = Parasitiformes; Murrell et al. 2001), the tick community cannot exploit fully the available genome sequences of insects, such as *Drosophila melanogaster* (Meigen), and the rapid advances in our knowledge of the biology of insects. For example, the amino acid sequence from the *hsp70*, ferritin, and serpin coding regions of *Ixodes scapularis* Say and *Boophilus microplus* (Canestrini) is 58, 84, and 34% identical, respectively. Using the *I. scapularis* sequence as the only tick model for gene discovery efforts could be very problematic in many experimental designs.

Increased sequence information on *B. microplus* should provide insight on how the tick develops acaricide resistance and should lead to better means of delaying and circumventing resistance problems. More efficient control of *B. microplus* will benefit agricultural producers and consumers worldwide through reduced expenditures for tick control. It also is anticipated that genome sequence information will guide the development of novel tick control methodologies that are more specific and of less mammalian toxicity than currently used chemical controls. Historically, use of chemical acaricides to control ticks has led to environmental contamination, particularly around cattle treatment sites, as well as negative impacts on consumer acceptance of meat and international trade in cattle products because of chemical residues from tick treatment. New means of tick control are needed to minimize these issues.

Sequence information would assist studies seeking to identify genes involved in pathogen acquisition, maintenance, and transmission, particularly with the agriculturally important tick disease complex of *Boophilus* spp.–*Babesia* spp.–*Anaplasma marginale.* An increased understanding of this complex should be beneficial to other studies of host–pathogen relationships, including those involving mosquito and tick species of human health importance.

Advancing the Knowledge of Human Biology. When a vertebrate host is bitten by an infected tick, pathogens are transmitted to the host animal via tick saliva. *B. microplus* can transmit bacteria, viruses, and protozoa, several of which are pathogenic. Additionally, the tick's saliva contains many components that play roles in affecting the host's immunological, hemostatic, and inflammatory pathways and that allow the tick to successfully complete feeding (Wikel 1999; Turni et al. 2002, 2004). Although the immunomodulation of host response by tick salivary proteins is well known, effects on the transmission and persistence of various tick-borne pathogens is not well understood. Ticks also are capable of regenerating amputated limbs and associated sensory organs after a single molt (Leonovich and Belozerov 1992). In fact, *B. microplus* has been shown to continue feeding and to retain a functional feeding apparatus while undergoing molting in other parts of its body (Jorgensen and Kemp 1986). The genes involved in these regenerative processes are unknown, as are genes that regulate or guide transitions through other developmental stages. The genome sequence should facilitate identification of the genes involved in the tick's ability to evade host responses to the tick bite and genes involved in its regenerative process. Comparative genomic studies that incorporate the information about these tick genes should advance the knowledge of genes regulating human immune responses, development, and tissue regeneration.

The apparent simplicity of the life cycle of *B. microplus* belies its genome size that is several times larger than the human genome. This disparity between organism complexity and genome size is puzzling and a comparative genomics approach should provide information about essential elements of the genomes of higher eukaryotes.

Expanding Our Understanding of Basic Biological Processes. *B. microplus,* as a one-host tick, is closely associated with its bovine host, with female ticks leaving the animal only for oviposition. Genome information will enhance the understanding of the evolution of the dependence on a single host at all life stages. With its dependence on a single host, *B. microplus* is vulnerable to problems associated with host availability and host immune responses. Yet, this specialization may enhance the organism's success because genomic resources need focus on overcoming only a single host's responses to the tick bite. Multihost ticks, such as *I. scapularis,* likely require different strategies to evade responses from each host and probably experience greater ranges of host responses than the one-host ticks. Again, this brings up the interesting question centering on why *B. microplus,* an organism with a seemingly simple life cycle, requires such a large genome. Tick genome sequence and gene identification will facilitate understanding of molecular mechanisms underlying some extremely interesting aspects of tick biology regarding their hardiness and longevity. Although vulnerable to desiccation, ticks are able to survive harsh environmental conditions of high temperatures and high humidity and can be very long lived. There is documentation of tick eggs hatching after 80 d of submersion and adults surviving 3 wk of submersion. Some species of the soft-bodied ticks can survive starvation conditions for up to 16 yr. Finally, an ixodid species of tick has a recorded total life cycle of >21 yr (James and Harwood 1969).

Availability of the *B. microplus* genome sequence, in conjunction with the ongoing genome projects for the cow, *Bos taurus,* and its pathogens *Babesia bovis, Babesia bigemina,* and *Anaplasma marginale,* will provide extraordinary opportunity for studies to discern the molecular basis of host–vector–pathogen interactions. Genome sequences would be available for all three foci in the host–tick–pathogen complex involving *B. bovis, B. bigemina,* and *A. marginale.* Little is known about genes involved in regulating the tick's defenses against bovine immune responses to the tick bite or genes critical to the initial infection of the tick by pathogenic microorganisms and subsequent propagation within the tick and finally transmission to the mammalian host. Ticks harbor many microorganisms, both symbiotic and pathogenic, and the relationship between the tick and theses microbes is poorly understood. Understanding this process in *B. microplus* should be beneficial to studies of other arthropods that transmit pathogens of agricultural or medical impact, such as *I. scapularis, Anopheles gambiae* Giles, *Culex pipiens* L., and tsetse (*Glossina* spp.).

Providing Additional Surrogate Systems for Experimentation. RNA interference studies have been successful in ticks (Aljamali et al. 2003), and this technique promises to be significant in assessing function and role of tick gene products and will be particularly well-suited for dissecting host–tick–pathogen interactions at the gene level. Genome sequence information will enhance both the ability to identify gene products critical to those interactions and to identify candidates for RNA interference (RNAi) studies seeking to identify genes playing central roles in pathogen transmission and in host immune response to the tick bite and pathogen infection. Insight will be gained on the regulation of biological processes in the tick and the specific genes and pathways involved.

Additionally, because strains of *B. bovis, B. bigemina,* and *A. marginale* can be maintained in culture, these experimental tools are available to couple with available *B. microplus* cell lines or tick strains to design experimental systems to test interesting biological questions.

Positive Impacts on the Ability to Do Experiments in Other Organisms. The utility of the genome sequence for studies of tick– host–pathogen interactions has been discussed above. The discovery of genes critical to those interactions and those relating to vector competence should provide targets for development of tick control strategies based on inhibiting pathogen transmission. The *B. microplus* genome sequence will facilitate comparative genomics studies with other arthropods, including other disease vectors of agricultural and medical importance. The genome sequence of *B. microplus* will help identify and interpret gene organization, function, and regulation in other arachnids through comparative bioinformatics approaches.

A significant amount of research has examined the development of pesticide resistance in *B. microplus.* Single base mutations that lead to pesticide resistance have been identified in *B. microplus* genes (He et al. 1999, Guerrero et al. 2002). The *B. microplus* genome will enable the identification of the full complement of genes from the organism and the regulatory regions governing their expression. This should lead to identification of novel pesticide target genes whose study would be most feasible in *B. microplus* compared with other arachnids, given the already significant knowledge about pesticide action and metabolism in this species. Identification of gene regulatory regions and codon usage in both highly and rarely expressed genes of *B. microplus* also will be important in constructing appropriate gene vectors for developing stably transformed transgenic tick strains or in vitro cell lines. Although studies in *B. microplus* cell lines have been reported (Cossio-Bayugar et al. 2002, Herron et al. 2004), optimization of transformation protocols will require information about gene promoters and codon usage that can be supplied by a genome project. Additionally, the genome project will allow the identification of low copy number genes that are very difficult to identify through expressed sequence tag (EST)-based projects. Some of these types of genes include those involved in chemoreception, host competency, pesticide binding, and transcription regulation.

Strategic Issues Critical to the Decision to Acquire New Sequence Data

Demand for *B. microplus* Genome Sequence. An active research community is involved in study of ticks and related organisms. The *B. microplus* genome sequence will be a valuable database for laboratories that study arthropods in general, ticks specifically, vector biology, host–vector–pathogen interactions, and the agricultural community, including researchers in the chemical pesticide industry. There were >100 independent researchers on the distribution list of this article as it was drafted and many contributed to its final form. There are ≈300 members of the Systematic and Applied Acarology Society and 2,531 acarologists listed in the Directory of Acarologists of the World 2002 (http://www.nhm.ac.uk/hosted_sites/acarology/ica/directory). More than 170 researchers attended the fourth International Conference on Ticks and Tick-Borne Pathogens (TTP-4) held in Banff, Alberta, Canada, in July 2002. Specific interest in the *B. microplus* genome project was prominent in three internationally attended meetings dedicated to tick genomics. These meetings were 1) the "Workshop on Tick Genomics" conducted at the 4th Annual International Conference on Ticks and Tick-Borne Pathogens held in Banff in July 2002; 2) the "Working Group on Tick Genomics" meeting at The Institute for Genome Research (TIGR) cosponsored by the U.S. Department of Agriculture held in Rockville, MD, in February 2003; and 3) the "Tick Genomics and the Future of Tick Research" meeting held in Baltimore, MD, in May 2004. A much larger community, including those conducting mammalian studies, will use *B. microplus* genome sequences to enhance their understanding of gene function, expression, and regulation in their organism of interest. Comparative genomics is proving to be an extraordinarily powerful approach in the post-genomic era. The present lack of a genome sequence from the Metastriata lineage of ticks restrains comparative studies of ticks, arthropods, and Metazoa. The Metastriata contains most of the ticks of veterinary and medical importance, e.g., the species of *Boophilus, Rhipicephalus, Hyalomma, Hemaphysalis,* and *Amblyomma* (Barker and Murrell 2004). The genome sequence of *B. microplus* will fill this gap. Once the *B. microplus* genome is sequenced, both of the two major lineages of hard ticks (Ixodidae) will be represented: *I. scapularis* from the Prostriata (project funded and in progress) and *B. microplus* from the Metastriata.

A partial list of biological resources or data that serve as the foundation to the current state of the *B. microplus* genome project follows:

1. Tick colonies for biological materials used in purification of genomic DNA and mRNA for cDNA library synthesis (Drs. Ron B. Davey and Robert J. Miller, USDA–ARS Cattle Fever Tick Research Laboratory, Moore Field, TX).
2. *B. microplus* genome size determination by C_oT analysis (A. Ullmann, Centers for Disease Control and Prevention, Ft. Collins, CO, and Dr. W. C. Black IV, Colorado State University, Ft. Collins, CO).
3. *B. bovis*-infected *B. microplus* ticks and database of *B. microplus* pathogens (Drs. Don Knowles and Glen Scoles, USDA–ARS Animal Disease Research, Pullman, WA).
4. *B. microplus* cytogenetics, including assignment of specific bacterial artificial chromosome (BAC) and cDNA sequences to specific *B. microplus* chromosomes (Drs. Catherine Hill and Jeff Stuart, Purdue University, West Lafayette, IN).
5. Normalized *B. microplus* cDNA library used to obtain the *B. microplus* gene index of >45,000 sequenced ESTs (Dr. Christian Gruber, Express Genomics, Frederick, MD).
6. *B. microplus* tick cell line for use in cytogenetic mapping and RNAi studies (Dr. T. Kurtti, University of Minnesota, St. Paul, MN).
7. *B. microplus* gene index (BmiGI http://www.tigr.org) containing annotated database of *B. microplus* ESTs, including Blast and gene ontology bioinformatic analyses (Dr. Vish. Nene, The Institute for Genomic Research, Rockville, MD).

8. Acaricide resistant and susceptible isolates of *B. microplus* and *Babesia* stocks (Drs. Wayne Jorgensen, Ala Lew, and Louise Jackson, Department of Primary Industries and Fisheries, Yeerongpilly, Queensland, Australia).

Suitability of *B. microplus* for Experimentation. Laboratories in Africa, Brazil, Mexico, Australia, and the United States maintain a number of unique strains of *B. microplus* colonized on cattle. Depending on environmental conditions, four to five generations per year can be obtained. The strain Deutsch, which was selected to provide genomic DNA for C_0t genome size analysis and BAC and genomic DNA library synthesis, is maintained at the USDA–ARS Cattle Fever Tick Research Laboratory in Mission, TX. The colony was derived from ticks collected on October 2001 from an outbreak in Webb County TX, has been inbred in the laboratory for 12 generations without noticeable loss of viability and can be rapidly expanded to provide material from any life stage for analysis. *B. microplus* seems to lose viability only very slowly as Australian strains have been maintained for many years longer than the Deutsch strain without loss of viability. To ensure strain security, a second location will be selected to maintain the Deutsch strain.

B. microplus contains 10 pairs of autosomes and one X-chromosome in males and two X-chromosomes in females (Hilburn et al. 1989). Although little information has been reported on genetic or physical markers for this tick, a collaborative effort between the laboratories of Drs. Hill, Stuart, and Guerrero is underway. The specific aim of that project is to use fluorescent in situ hybridization methodology (Brown et al. 2001) to identify each of the chromosomes of *B. microplus* and assign specific markers (cDNAs or BACs) to positions on each chromosome. These mapped markers will be useful for assembling the genome sequence, genetic studies for predicting gene locations and for selecting candidate genes for traits of interest. The 7.1-Gbp genome size of *B. microplus* was determined using ticks from the Deutsch strain and consists of 30% unique, 38% moderately repetitive, 31% highly repetitive, and 0.82% foldback DNA (Ullmann et al. 2005). Most of the DNA follows a pattern of short period interspersion, although repetitive sequences occur in a mixture of long and short period interspersion. The highly repetitive fraction of the genomic DNA is of very low complexity (≈153,000 copies/haploid genome), which presents an advantage in searching genomic sequence for likely gene coding regions but becomes disadvantageous when assembling sequences into longer contigs. There are techniques available to enrich genomic DNA for unique fragments. These approaches may help the sequence assembly in gene coding regions by obtaining more sequence from these areas.

Gene silencing through RNAi has been demonstrated in *Amblyomma americanum* (L.) (Aljamali et al. 2003), *Hemaphysalis longicornis* (Miyoshi et al. 2004), and *I. scapularis* (Narasimhan et al. 2004) and is expected to function in *Boophilus* ticks as well. Coupled with availability of the *B. microplus* genome sequence, this technique will be extremely valuable in functional genomics studies of this organism both in vivo and in the available tick cell lines described previously.

Presently, a *B. microplus* BAC library is available and consists of ≈1X coverage with average insert size of 118 kb. The BAC library was synthesized before the availability of the *B. microplus* genome size data (Ullmann et al. 2005), and clone selection was based on the one Gbp genome size estimate for *A. americanum* (Palmer et al. 1994). Plans are underway to expand the coverage of the BAC library to at least 5X.

A *B. microplus* gene index (BmiGI; Guerrero et al. 2005) has been developed in a collaborative USDA–TIGR project and is available at the TIGR Web site (http://www.tigr.org/tdb/tgi). The current version of BmiGI consists of 8,270 unique sequences derived from 20,417 EST sequences, and ≈25,000 new ESTs have been sequenced and are being annotated for inclusion in BmiGI.

Rationale for Obtaining the Sequence of the Organism. The goal of the *B. microplus* genome project is the attainment of a draft sequence for *B. microplus* of sufficient coverage and quality to facilitate studies in areas such as gene discovery, comparative genomics, functional genomics, vector biology, immunology, and so on leading to the development of novel tick control technologies. Fiscal constraints with current sequencing costs will not allow a final polished version of the genome sequence to be attained. In fact, the development of novel control technologies can be achieved without a completed genome. However, a genome coverage of 4–6X would be a great enhancement to worldwide research projects on this economically important agricultural pest and identify many more potential control targets than EST-based approaches can provide. It is expected the genome sequence will identify most of the genes of *B. microplus*, including gene regulatory regions, providing information that could lead to genetic manipulation of these ticks. This could include transposable elements, promoter sequences for gene expression, and coding regions useful for creation of conditional lethal or sterile lines. The value of the funded *I. scapularis* genome sequence project will be enhanced by the availability of the *B. microplus* sequence, particularly facilitating comparative studies whose objective is the design of tick specific vaccines or control agents. The *B. microplus* project should facilitate the identification of tick-specific genes and single/low copy number genes that would be very difficult to detect in EST-based projects, including genes involved in regulatory cascades, G protein coupled receptors, chemoreceptors, acetylcholinesterase, and others. As in other fields of biology, great advances in understanding can be anticipated from the application of proteomics to ticks and the tick–host interaction. Given the paucity of protein sequence data for *B. microplus*, the feasible route to proteomic studies is via a genome sequence. The proteomics approach will be facilitated with an available *B. microplus* genome sequence that will enhance proteomic approaches by using mass spectrometry-assisted protein identifications.

Readiness of the Organism's DNA for Sequencing. The BAC library has been synthesized and additional colonies will be picked to increase genome coverage. Sequencing of two randomly chosen and four selected BAC clones has begun and should provide information about genome organization in *B. microplus.* Genomic DNA has been isolated and can be used for synthesis of genomic libraries of low, moderate, and large insert size. These libraries would be available for whole genome shotgun sequencing strategies at the sequencing center selected for conducting the *B. microplus* project. Eggs and larvae from the selected strain of *B. microplus* have been accumulated over several generations and are available for purification of additional genomic DNA if needed. Additionally, the egg and larval production from the colony can be increased as necessary. The EST sequencing project at TIGR has produced sequence from ≈45,000 ESTs from a normalized cDNA library synthesized from various life stages and tissues of *B. microplus* and compiled into the *B. microplus* gene index and the first version is available at www.tigr.org. As significant numbers of new ESTs are analyzed, the gene index will be updated. Several full-length cDNAs have been identified in the gene index and plans are in place to complete sequencing of at least 100 full-length cDNAs. These sequences will assist in the chromosome mapping collaboration between Agricultural Research Service and Purdue University and also as resources for training gene finding programs to identify tick gene sequences. The size of the *B. microplus* genome and the presence of large amounts of repetitive DNA (Ullmann et al. 2005) will present challenges to the assembly of the draft genome sequence. The whole genome shotgun sequencing strategy will be supplemented by data from extensive BAC end sequencing that will be used to help assemble contigs into scaffolds. The BACs and cDNAs that have been assigned chromosome locations from cytogenetic studies should prove useful for assigning scaffolds to specific chromosomes. It is anticipated that genome sequence information generated by publicly funded portions of this project will be disseminated soon after each assembly and freely available on a Web site dedicated for this purpose. The selected sequencing center will be responsible for automated sequence assembly and preliminary annotation of the *B. microplus* genome. Because the 4–6X draft coverage we are proposing will not produce a finished sequence, gap closure will likely be dependent upon independently supported groups with interests in specific regions of the genome. The BAC end sequences and the six completely sequenced BACs also will provide information to train gene finding/annotation programs for tick-specific DNA sequence characteristics. The availability of the *I. scapularis* genome sequence also will assist the assembly and annotation of the *B. microplus* genome.

Ongoing Complementary Projects

USDA-ARS is currently supporting research which will facilitate a *B. microplus* genome project:

1. ARS Project No. 6205-32000-026-00D, Knipling–Bushland U.S. Livestock Insects Research Laboratory, duration 1 June 2004–31 May 2009, entitled "Molecular Biology of *Boophilus microplus.*"
2. Assistance Type Cooperative Agreement 58-6205-4-007 between ARS–Kerrville (Dr. Guerrero) and TIGR (Dr. Nene), duration 1 May 2004–30 April 2009, entitled "An Investigation of the Genome Structure of the Tick, *Boophilus microplus,* and the Development of a Database to Support a Tick Genome Project."
3. Specific Cooperative Agreement 58-6205-4-008 between ARS–Kerrville (Dr. Guerrero) and Purdue University (Dr. Hill), duration 15 September 2004–30 September 2006, entitled "Cytogenetics for *Boophilus microplus* genomics."
4. USDA–CSREES National Research Initiative Competitive Grants Program 45.0 Functional Genomics of Agriculturally Important Organisms Funded Proposal #04-148-5301, Project director Dr. Kelly Brayton (Washington State University), coproject directors Drs. Guy Palmer (Washington State University) and Felix Guerrero (USDA–ARS), duration 1 April 2005–31 March 2009, entitled "Functional genomics of the tick vector-pathogen interface" to investigate the functional genomics of *B. microplus–Anaplasma marginale* interactions by using the completed sequence of the *A. marginale* genome and the EST database and available genomic resources of *B. microplus.*

The level of funding and interest by USDA establishes the critical nature of the potential impact which reinfestation of the United States by *B. microplus* would have on U.S. agriculture. Additionally, preliminary discussions are underway to establish a collaboration between Brazilian and U.S. scientists regarding screening novel *B. microplus* genes as antigens for antitick vaccine development. Tissue- and stage-specific cDNA libraries will be used as a resource for high throughput EST sequencing. Candidate genes will be identified through bioinformatic approaches and screened for antigenicity using T- and B-cell expression cloning techniques. This collaboration may open Brazilian federal and state agencies as potential partners in funding this tick genome project.

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